	L #	Hits	Search Text
1	L1	1346	glycosidase$2
2	L2	3660	beta adj 2 1
3	L3	35	thermophillic
4	L4	6145	100 adj2 c
5	L5	35	2 and 4
6	L6	157	pyrococcus
7	L7	0	5 and 6
8	L8	1	ph adj3 6
9	L9	79	beta adj2 glycosidase$2
10	L10	4	9 and 4
11	L11	4744	4 and temperature
12	L13	0	12 and 6
13	L12	32	2 and 11
14	L14	10	12 and optimum

[redacted] activity at pH 7.4 and 105 degree C. Although the enzyme had a half-life of [redacted] greater than 60 h at 90 degree C, it is much less thermostable than the beta -glucosidase, which had a reported half-life of 85 h at 100 degree C. K sub(m) and V sub(max) values for the beta -mannosidase were determined to be 0.79 mM and 31.1 mu mol para-nitrophenol released/min/mg with p-nitrophenyl- beta -D-mannopyranoside as substrate. The catalytic efficiency of the beta -mannosidase was significantly lower than that reported for the P. furiosus beta -glucosidase (5.3 versus 4, 500 s super(-1) mM super(-1) with p-nitrophenyl- beta -D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta -glucosidase, the primary role of the beta -mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

=> d his

(FILE 'HOME' ENTERED AT 08:37:47 ON 10 MAY 2000)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 08:38:10 ON 10 MAY 2000

```
L1         95500 S GALACTOSIDASE?
L2         80870 S BETA(W)L1
L3           349 S B(W)L1
L4         81054 S L2 OR L3
L5            87 S THERMOPHILLIC
L6             1 S L5 AND (L1 OR L4)
L7            86 S L1 AND (100 (2W)C)
L8          3787 S PYROCOCCUS
L9             7 S L7 AND L8
L10            3 DUP REM L9 (4 DUPLICATES REMOVED)
L11            2 S L10 AND PH
L12        23070 S GLYCOSIDASE?
L13         1110 S BETA(W)L12
L14           65 S B(2W)L12
L15         1169 S L13 OR L14
L16            0 S L15 AND L5
L17            0 S L12 AND L5
L18           77 S L8 AND L12
L19           26 S L18 AND (100(2W)C)
L20           15 S L19 AND PH
```

=> dup rem

ENTER L# LIST OR (END):120

PROCESSING COMPLETED FOR L20

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L21            6 DUP REM L20 (9 DUPLICATES REMOVED)
```

=> d 1-6 ibib ab

L21 ANSWER 1 OF 6 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2000141228 MEDLINE

DOCUMENT NUMBER: 20141228

TITLE: Novel substrate specificity of a membrane-bound beta-**glycosidase** from the hyperthermophilic archaeon **Pyrococcus** horikoshii.

AUTHOR: Matsui I; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y; Honda K

CORPORATE SOURCE: National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp

SOURCE: FEBS LETTERS, (2000 Feb 11) 467 (2-3) 195-200.

L20 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:95843 HCAPLUS
TITLE: Novel substrate specificity of a membrane-bound .beta.-**glycosidase** from the hyperthermophilic archaeon **Pyrococcus** horikoshii
AUTHOR(S): Matsui, I.; Sakai, Y.; Matsui, E.; Kikuchi, H.; Kawarabayasi, Y.; Honda, K.
CORPORATE SOURCE: National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki, Japan
SOURCE: FEBS Lett. (2000), 467(2,3), 195-200
CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A .beta.-**glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully expressed in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85.degree.C for 15 min. The optimum **pH** was 6.0 and the optimum temp. was over **100**.degree.**C**, resp. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90.degree.C (**pH** 6.0) was 15 h. BGPh has a novel substrate specificity with kcat/Km values high enough for hydrolysis of .beta.-D-Glcp derivs. with long alkyl chain at the reducing end and low enough for the hydrolysis of .beta.-linked glucose dimer more hydrophilic than aryl- or alkyl-.beta.-D-Glcp.

late.

L20 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1997:170983 BIOSIS
DOCUMENT NUMBER: PREV199799477586
TITLE: A new thermostable glucose-activated beta-glucosidase from the hyperthermophilic marine archaebacterium **Pyrococcus** abyssi: Purification and characterization.
AUTHOR(S): Ladrat, Christine (1); Alayse-Danet, Anne-Marie; Dietrich, Jacques; Barbier, George
CORPORATE SOURCE: (1) Dep. Environ. Profond, IFREMER, BP 70, 29280 Plouzane France
SOURCE: Journal of Marine Biotechnology, (1996) Vol. 4, No. 4, pp. 192-199.
ISSN: 0941-2905.
DOCUMENT TYPE: Article
LANGUAGE: English

AB A thermostable beta-glucosidase from the deep-sea hyperthermophilic archaebacterium **Pyrococcus** abyssi strain ST549 has been purified to apparent homogeneity and characterized. Production of beta-glucosidase by this strain was stimulated by the addition of cellobiose to the culture medium. This enzyme was homodimeric, each subunit having a molecular weight of 64 kDa. Its isoelectric point was 4.3. The enzyme showed maximal activity at **pH** 6 and **100** degree **C** and was highly thermostable. It exhibited different beta-**glycosidase** activities and hydrolyzed short-chain oligosaccharides. Chemical modifications of amino acids indicated that no accessible sulfhydryl group was essential for activity and that histidine and tryptophan were probably involved in the maintenance of the integrity of the active site. In marked contrast to others presently known, the beta-glucosidase from the ST549 strain was activated by glucose and polyols. This property suggests specific interactions between the enzyme and polyhydric alcohols and the probable occurrence of a transglycosylation mechanism. This mechanism and potential applications are discussed.

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SESSION RESUMED IN FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH,
HCAPLUS,
NTIS, LIFESCI' AT 09:16:54 ON 10 MAY 2000
FILE 'MEDLINE' ENTERED AT 09:16:54 ON 10 MAY 2000
FILE 'EMBASE' ENTERED AT 09:16:54 ON 10 MAY 2000
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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    23.66      23.81

=> his

HIS IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s glycosidase?

   7 FILES SEARCHED...
L12      23070 GLYCOSIDASE?

=> d his


     (FILE 'HOME' ENTERED AT 08:37:47 ON 10 MAY 2000)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:38:10 ON 10 MAY 2000
L1         95500 S GALACTOSIDASE?
L2         80870 S BETA(W)L1
L3           349 S B(W)L1
L4         81054 S L2 OR L3
L5            87 S THERMOPHILLIC
L6             1 S L5 AND (L1 OR L4)
L7            86 S L1 AND (100 (2W)C)
L8          3787 S PYROCOCCUS
L9             7 S L7 AND L8
L10            3 DUP REM L9 (4 DUPLICATES REMOVED)
L11            2 S L10 AND PH
```

```
L12        23070 S GLYCOSIDASE?

=> s beta(w)l12

L13         1110 BETA(W) L12

=> s b(2w)l12

L14           65 B(2W) L12

=> s l13 or l14

L15         1169 L13 OR L14

=> s l15 and l5

L16            0 L15 AND L5

=> s l12 and l5

L17            0 L12 AND L5

=> s l8 and l12

L18           77 L8 AND L12

=> s l18 and (100(2w)C)

L19           26 L18 AND (100(2W) C)

=> s l19 and ph

   6 FILES SEARCHED...
L20           15 L19 AND PH

=> dup rem

ENTER L# LIST OR (END):l20

COMMAND INTERRUPTED
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help
Desk by telephone or via SEND in the STNMAIL file.

=> dup rem

ENTER L# LIST OR (END):l19

COMMAND INTERRUPTED
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help
Desk by telephone or via SEND in the STNMAIL file.

=> d his


     (FILE 'HOME' ENTERED AT 08:37:47 ON 10 MAY 2000)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:38:10 ON 10 MAY 2000
L1         95500 S GALACTOSIDASE?
L2         80870 S BETA(W)L1
L3           349 S B(W)L1
L4         81054 S L2 OR L3
L5            87 S THERMOPHILLIC
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L6            1 S L5 AND (L1 OR L4)
L7           86 S L1 AND [redacted]0 (2W)C)
L8         3787 S PYROCOCCUS
L9            7 S L7 AND L8
L10           3 DUP REM L9 (4 DUPLICATES REMOVED)
L11           2 S L10 AND PH
L12       23070 S GLYCOSIDASE?
L13        1110 S BETA(W)L12
L14          65 S B(2W)L12
L15        1169 S L13 OR L14
L16           0 S L15 AND L5
L17           0 S L12 AND L5
L18          77 S L8 AND L12
L19          26 S L18 AND (100(2W)C)
L20          15 S L19 AND PH

=> d 1-15 ibib ab


L20  ANSWER 1 OF 15  MEDLINE
ACCESSION NUMBER:   2000141228     MEDLINE
DOCUMENT NUMBER:    20141228
TITLE:              Novel substrate specificity of a membrane-bound beta-
                    glycosidase from the hyperthermophilic archaeon
                    Pyrococcus horikoshii.
AUTHOR:             Matsui I; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y;
                    Honda K
CORPORATE SOURCE:   National Institute of Bioscience and Human-Technology,
                    Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp
SOURCE:             FEBS LETTERS, (2000 Feb 11) 467 (2-3) 195-200.
                    Journal code: EUH. ISSN: 0014-5793.
PUB. COUNTRY:       Netherlands
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals; Cancer Journals
ENTRY MONTH:        200006
ENTRY WEEK:         20000602
AB   A beta-glycosidase gene homolog of Pyrococcus
     horikoshii (BGPh) was successfully expressed in Escherichia coli. The
     enzyme was localized in a membrane fraction and solubilized with 2.5%
     Triton X-100 at 85 degrees C for 15 min. The optimum pH was 6.0
     and the optimum temperature was over 100 degrees C,
     respectively. BGPh stability was dependent on the presence of Triton
     X-100, the enzyme's half-life at 90 degrees C (pH 6.0) was 15 h.
     BGPh has a novel substrate specificity with k(cat)/K(m) values high
enough
     for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the
     reducing end and low enough for the hydrolysis of beta-linked glucose
     dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp.

L20  ANSWER 2 OF 15  MEDLINE
ACCESSION NUMBER:   96394494     MEDLINE
DOCUMENT NUMBER:    96394494
TITLE:              Comparison of a beta-glucosidase and a beta-mannosidase
                    from the hyperthermophilic archaeon Pyrococcus
                    furiosus. Purification, characterization, gene cloning,
and
                    sequence analysis.
AUTHOR:             Bauer M W; Bylina E J; Swanson R V; Kelly R M
CORPORATE SOURCE:   Department of Chemical Engineering, North Carolina State
                    University, Raleigh, North Carolina 27695-7905, USA.
SOURCE:             JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Sep 27) 271 (39)
                    23749-55.
                    Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY:       United States
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DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Engli[redacted]
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-U60214
ENTRY MONTH: 199701

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557U37557). The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta-**glycosidases** from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at **pH** 7.4 and 105 degrees C. Although the enzyme had a half-life of greater than 60 h at 90 degrees C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at **100** degrees **C**. Km and Vmax values for the beta-mannosidase were determined to be 0.79 mM and

31.1 micromol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the P. furiosus beta-glucosidase (5.3 versus 4, 500 s-1 mM-1 with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 3 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000055777 EMBASE
TITLE: Novel substrate specificity of a membrane-bound .beta.-**glycosidase** from the hyperthermophilic archaeon **Pyrococcus** horikoshii.
AUTHOR: Matsui I.; Sakai Y.; Matsui E.; Kikuchi H.; Kawarabayasi Y.; Honda K.
CORPORATE SOURCE: I. Matsui, National Institute, Bioscience and Human Technology, Tsukuba, Ibaraki 305, Japan. ikmatsui@nibh.go.jp
SOURCE: FEBS Letters, (2000) 467/2-3 (195-200).
Refs: 32
ISSN: 0014-5793 CODEN: FEBLAL
PUBLISHER IDENT.: S 0014-5793(00)01156-X
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A .beta.-**glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully expressed in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85.degree.C for 15 min. The optimum **pH** was 6.0

and the optimum temperature was over **100**.degree.**C**, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90.degree.C (**pH** 6.0) was 15 h. BGPh has a novel substrate specificity with k(cat)/K(m) values high enough for hydrolysis of .beta.-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of .beta.-linked glucose dimer more hydrophilic than aryl- or alkyl-.beta.-D-Glcp. Copyright (C) 2000 Federation of European Biochemical Societies.

L20 ANSWER 4 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999327626 EMBASE
TITLE: Gene analysis and enzymatic properties of thermostable .beta.-**glycosidase** from **Pyrococcus** kodakaraensis KOD1.
AUTHOR: Ezaki S.; Miyaoku K.; Nishi K.-I.; Tanaka T.; Fujiwara S.; Takagi M.; Atomi H.; Imanaka T.
CORPORATE SOURCE: T. Imanaka, Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
SOURCE: Journal of Bioscience and Bioengineering, (1999) 88/2 (130-135).
Refs: 22
ISSN: 1389-1723 CODEN: JBBIF6
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A .beta.-**glycosidase** with broad substrate specificity was identified from a hyperthermophilic archaeon, **Pyrococcus** kodakaraensis KOD1. The gene encoding .beta.-**glycosidase** (Pk-gly) consists of 1449 nucleotides corresponding to a polypeptide of 483 amino acids. The protein showed similarity with other .beta.-**glycosidases** from family-1 glycosyl hydrolases, in particular, it showed high identity to .beta.-mannosidase from P. furiosus (55.7%), .beta.-**glycosidase** from Sulfolobus solfataricus (42.7%) and .beta.-glucosidase from P. furiosus (41.9%). The cloned gene was expressed in Escherichia coli and the recombinant protein was purified. The .beta.-**glycosidase** showed optimal activity at **pH** 6,5 and at an extremely high temperature of **100**.degree.**C**, and had a half-life of 18 h at 90.degree.C. The .beta.-**glycosidase** hydrolyzed various pNp-.beta.-glycopyranosides, with k(cat)/K(m) values in the order of pNp-.beta.-glucopyranoside dot left right equal to pNp-.beta.-mannopyranoside>pNp-.beta.-galactopyranoside>pNp-.beta.-xylopyranoside. pNp-.beta.-mannopyranoside was the substrate exhibiting the lowest K(m) value [0.254 mM] with a k(cat)/K(m) ratio comparable to that of pNp-.beta.-glucopyranoside. This substrate specificity was distinct from previously reported .beta.-**glycosidases**. We observed that the region in Pk-Gly corresponding to the fifth .alpha.-helix and .beta.-strand region of .beta.-**glycosidase** from S. solfataricus, which constitutes a large portion of the channel for substrate incorporation, displayed a chimeric structure, with the N-terminal region similar to .beta.-**glycosidases** and the C-terminal region similar to .beta.- mannosidases. An exo-type hydrolytic activity and transglycosylation activity were also observed towards cellooligomers.

L20 ANSWER 5 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97196266 EMBASE

DOCUMENT NUMBER: 1997182266
TITLE: Cloning, sequencing, characterization, and expression of an extracellular .alpha.-amylase from the hyperthermophilic archaeon **Pyrococcus** furiosus in Escherichia coli and Bacillus subtilis.
AUTHOR: Jorgensen S.; Vorgias C.E.; Antranikian G.
CORPORATE SOURCE: G. Antranikian, Inst. of Biotechnology, Dept. of Technical Microbiology, Technical University Hamburg-Harhurg, Denickestr. 15, 21071 Hamburg, Germany
SOURCE: Journal of Biological Chemistry, (1997) 272/26 (16335-16342).
Refs: 27
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A gene encoding a highly thermostable extracellular .alpha.-amylase from the hyperthermophilic archaeon **Pyrococcus** furiosus was identified. The gene was cloned, sequenced, and expressed in Escherichia coli and Bacillus subtilis. The gene is 1383 base pairs long and encodes a protein of 461 amino acids. The open reading frame of the gene was verified by microsequencing of the recombinant purified enzyme. The deduced amino acid sequence is 25 amino acids longer at the N terminus than that determined by sequencing of the purified protein, suggesting that a leader sequence is removed during transport of the enzyme across the membrane. The recombinant .alpha.-amylase was biochemically characterized and shows an activity optimum at **pH** 4.5, whereas the optimun temperature for enzymatic activity is close to **100** .degree.**C**. .alpha.- Amylase shows sequence homology to the other known .alpha.-amylases and belongs to family 13 of glycosyl hydrolases. This extracellular .alpha.-amylase is not homologous to the subcellular .alpha.-amylase previously isolated from the same organism.

L20 ANSWER 6 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96300283 EMBASE
DOCUMENT NUMBER: 1996300283
TITLE: Comparison of a .beta.-glucosidase and a .beta.-mannosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus. Purification, characterization, gene cloning, and sequence analysis.
AUTHOR: Bauer M.W.; Bylina E.J.; Swanson R.V.; Kelly R.M.
CORPORATE SOURCE: Dept. of Chemical Engineering, North Carolina State University,Raleigh, NC 27695-7905, United States
SOURCE: Journal of Biological Chemistry, (1996) 271/39 (23749-23755).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Two distinct exo-acting, .beta.-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a .beta.-glucosidase, corresponding to the one previously purified by Kengen et al (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213,

305-312), and a.beta. mannosidase. The .beta.-mannosidase and .beta.-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amine acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the .beta.-mannosidase and the .beta.- glucosidase, respectively. The .beta.-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557). The deduced amine acid sequences showed hemology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the .beta.-**glycosidases** from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the .beta.-mannosidase and the .beta.-glucosidase can both be classified as family I glycosyl hydrolases. In addition, the .beta.-mannosidase and .beta.-glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The .beta.-mannosidase showed optimal activity at **pH** 7.4 and 105 .degree.C. Although the enzyme had a half-life of greater than 60 h at 90 .degree.C, it is much less thermostable than the .beta.- glucosidase, which had a reported half-life of 85 h at **100** .degree.**C**. Km and V(max) values for the .beta.-mannosidase were determined to be 0.79 mM and 31.1 .mu.mol para-nitrophenol released/min/mg with p-nitrophenyl-.beta.-d-mannopyranoside as substrate. The catalytic efficiency of the .beta.-mannosidase was significantly lower than that reported for the P. furiosus .beta.-glucosidase (5.3 versus 4, 500 s-1 mM-1 with p-nitrophenyl-B-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the .beta.-glucosidase, the primary role of the .beta.-mannosidase may not he disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 7 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 90219204 EMBASE
DOCUMENT NUMBER: 1990219204
TITLE: Purification and characterization of an .alpha.-glucosidase from a hyperthermophilic archaebacterium, **Pyrococcus** furiosus, exhibiting a temperature optimum of 105 to 115.degree.C.
AUTHOR: Costantino H.R.; Brown S.H.; Kelly R.M.
CORPORATE SOURCE: Department of Chemical, Engineering, Johns Hopkins University,Baltimore, MD 21218, United States
SOURCE: Journal of Bacteriology, (1990) 172/7 (3654-3660). ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Pyrococcus** furiosus is a strictly anaerobic hyperthermophilic archaebacterium with an optimal growth temperature of about **100** .degree.**C**. When this organism was grown in the presence of certain complex carbohydrates, the production of several amylolytic enzymes was noted. These enzymes included an .alpha.-**glycosidase** that was located in the cell cytoplasm. This .alpha.-glucosidase has been purified 310-fold and corresponded to a protein band of 125 kilodaltons as resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme exhibited optimum activity at **pH** 5.0 to 6.0 and over a temperature range of 105 to 115.degree.C. Kinetic analysis conducted at 108.degree.C revealed hydrolysis of the substrate p-nitrophenyl-.alpha.-D-

glucopyranoside (PNPG), methyl-.alpha.-D-glucopyranoside, maltose, and isomaltose. Trace activity was detected towards p-nitrophenyl-.beta.-D-glucopyranoside, and no activity could be detected towards starch or sucrose. Inhibition studies conducted at 108.degree.C with PNPG as the substrate and maltose as the inhibitor yielded a K(i) for maltose of 14.3 mM. Preincubation for 30 min at 98.degree.C in 100 mM dithiothreitol and 1.0 M urea had little effect on enzyme activity, whereas preincubation in 1.0% sodium dodecyl sulfate and 1.0 M guanidine hydrochloride resulted in significant loss of enzyme activity. Purified .alpha.-**glycosidase** from P. furiosus exhibited remarkable thermostability; incubation of the enzyme at 98.degree.C resulted in a half life of nearly 48 h.

L20 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1997:170983 BIOSIS
DOCUMENT NUMBER: PREV199799477586
TITLE: A new thermostable glucose-activated beta-glucosidase from the hyperthermophilic marine archaebacterium **Pyrococcus** abyssi: Purification and characterization.
AUTHOR(S): Ladrat, Christine (1); Alayse-Danet, Anne-Marie; Dietrich, Jacques; Barbier, George
CORPORATE SOURCE: (1) Dep. Environ. Profond, IFREMER, BP 70, 29280 Plouzane France
SOURCE: Journal of Marine Biotechnology, (1996) Vol. 4, No. 4, pp. 192-199.
ISSN: 0941-2905.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A thermostable beta-glucosidase from the deep-sea hyperthermophilic archaebacterium **Pyrococcus** abyssi strain ST549 has been purified to apparent homogeneity and characterized. Production of beta-glucosidase by this strain was stimulated by the addition of cellobiose to the culture medium. This enzyme was homodimeric, each subunit having a molecular weight of 64 kDa. Its isoelectric point was 4.3. The enzyme showed maximal activity at **pH** 6 and **100** degree **C** and was highly thermostable. It exhibited different beta-**glycosidase** activities and hydrolyzed short-chain oligosaccharides. Chemical modifications of amino acids indicated that no accessible sulfhydryl group was essential for activity and that histidine and tryptophan were probably involved in the maintenance of the integrity of the active site. In marked contrast to others presently known, the beta-glucosidase from the ST549 strain was activated by glucose and polyols. This property suggests specific interactions between the enzyme and polyhydric alcohols and the probable occurrence of a transglycosylation mechanism. This mechanism and potential applications are discussed.

L20 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1996:508084 BIOSIS
DOCUMENT NUMBER: PREV199699230440
TITLE: Comparison of a beta-glucosidase and a beta-mannosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus: Purification, characterization, gene cloning and sequence analysis.
AUTHOR(S): Bauer, Michael W.; Bylina, Edward J.; Swanson, Ronald V.; Kelly, Robert M. (1)
CORPORATE SOURCE: (1) Dep. Chem. Engineering, North Carolina State Univ., Raleigh, NC 27695-7905 USA
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 39, pp. 23749-23755.
ISSN: 0021-9258.

DOCUMENT TYPE: Article
LANGUAGE: English

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557). The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta-**glycosidases** from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at **pH** 7.4 and 105 degree C. Although the enzyme had a half-life of greater than 60 h at 90 degree C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at **100** degree **C**. K-m and V-max values for the beta-mannosidase were determined to be 0.79 mM and 31.1 mu-mol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the P. furiosus beta-glucosidase (5.3 versus 4,500 s-1 mM-1 with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 10 OF 15 SCISEARCH COPYRIGHT 2000 ISI (R)
ACCESSION NUMBER: 2000:156669 SCISEARCH
THE GENUINE ARTICLE: 285ZF
TITLE: Novel substrate specificity of a membrane-bound beta-**glycosidase** from the hyperthermophilic archaeon **Pyrococcus** horikoshii
AUTHOR: Matsui I (Reprint); Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y; Honda K
CORPORATE SOURCE: NATL INST BIOSCI & HUMAN TECHNOL, TSUKUBA, IBARAKI 305, JAPAN (Reprint); MINIST INT TRADE & IND, NATL INST TECHNOL & EVALUAT, TOKYO, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: FEBS LETTERS, (11 FEB 2000) Vol. 467, No. 2-3, pp. 195-200

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0014-5793.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE

LANGUAGE: English
REFERENCE COUNT: 32

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A beta-**glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully expressed in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85 degrees C for 15 min, The optimum **pH** was 6.0 and the optimum temperature was over **100** degrees **C**, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90 degrees C (**pH** 6.0) was 15 h, BGPh has a novel substrate specificity with k(cat)/K-m values high enough for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of beta-linked glucose dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp (C) 2000 Federation of European Biochemical Societies.

L20 ANSWER 11 OF 15 SCISEARCH COPYRIGHT 2000 ISI (R)
ACCESSION NUMBER: 1999:834444 SCISEARCH
THE GENUINE ARTICLE: 249NG
TITLE: Gene analysis and enzymatic properties of thermostable-beta-**glycosidase** from **Pyrococcus** kodakaraensis KOD1
AUTHOR: Ezaki S; Miyaoku K; Nishi K I; Tanaka T; Fujiwara S; Takagi M; Atomi H; Imanaka T (Reprint)
CORPORATE SOURCE: KYOTO UNIV, GRAD SCH ENGN, DEPT SYNTHET CHEM & BIOL CHEM, SAKYO KU, YOSHIDA HONMACHI, KYOTO 6068501, JAPAN (Reprint); KYOTO UNIV, GRAD SCH ENGN, DEPT SYNTHET CHEM & BIOL CHEM, SAKYO KU, KYOTO 6068501, JAPAN; OSAKA UNIV, GRAD SCH ENGN, DEPT BIOTECHNOL, SUITA, OSAKA 5650871, JAPAN; KANEKA CORP, FINE CHEM RES LABS, TAKASAGO, HYOGO 6768688, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOSCIENCE AND BIOENGINEERING, (AUG 1999) Vol. 88, No. 2, pp. 130-135.
Publisher: SOC BIOSCIENCE BIOENGINEERING JAPAN, OSAKA UNIV, FACULTY ENGINEERING, 2-1 YAMADAOKA, SUITA, OSAKA 565-0871, JAPAN.
ISSN: 1389-1723.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 22

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A beta-**glycosidase** with broad substrate specificity was identified from a hyperthermophilic archaeon, **Pyrococcus** kodakaraensis KOD1. The gene encoding beta-**glycosidase** (Pk-gly) consists of 1449 nucleotides corresponding to a polypeptide of 483 amino acids. The protein showed similarity with other beta-**glycosidases** from family-1 glycosyl hydrolases, in particular, it showed high identity to beta-mannosidase from P. furiosus (55.7%), beta-**glycosidase** from Sulfolobus solfataricus (42.7%) and beta-glucosidase from P. furiosus (41.9%). The cloned gene was expressed in Escherichia coli and the recombinant protein was purified. The beta-**glycosidase** showed optimal activity at **pH** 6.5 and at an extremely high temperature of **100** degrees **C**, and had a half-life of 18 h at 90 degrees C. The beta-**glycosidase** hydrolyzed various pNp-beta-glycopyranosides, with k(cat)/K-m values in the order of pNp-beta-glucopyranoside is approximately equal to pNp-beta-mannopyranoside > pNp-beta-galactopyranoside > pNp-beta-xylopyranoslde. pNp-beta-mannopyranoside was the substrate exhibiting the lowest K-m value [0.254 mM] with a k(cat)/K-m ratio comparable to that of pNp-beta-glucopyranoside. This substrate specificity was distinct from

previously reported beta-**glycosidases**. We observed that the region in Pk-Gly corresponding to the fifth alpha-helix and beta-strand region of beta-**glycosidase** from S. solfataricus, which constitutes a large portion of the channel for substrate incorporation, displayed a chimeric structure, with the N-terminal region similar to beta-**glycosidases** and the C-terminal region similar to beta-mannosidases. An exo-type hydrolytic activity and transglycosylation activity were also observed towards cellooligomers.

L20 ANSWER 12 OF 15 SCISEARCH COPYRIGHT 2000 ISI (R)
ACCESSION NUMBER: 96:698965 SCISEARCH
THE GENUINE ARTICLE: VJ442
TITLE: COMPARISON OF A BETA-GLUCOSIDASE AND A BETA-MANNOSIDASE FROM THE HYPERTHERMOPHILIC ARCHAEON **PYROCOCCUS** -FURIOSUS - PURIFICATION, CHARACTERIZATION, GENE CLONING, AND SEQUENCE-ANALYSIS
AUTHOR: BAUER M W; BYLINA E J; SWANSON R V; KELLY R M (Reprint)
CORPORATE SOURCE: N CAROLINA STATE UNIV, DEPT CHEM ENGN, RALEIGH, NC, 27695 (Reprint); N CAROLINA STATE UNIV, DEPT CHEM ENGN, RALEIGH, NC, 27695; RECOMBINANT BIOCATALYSIS INC, SHARON HILL, PA, 19079
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (27 SEP 1996) Vol. 271, No. 39, pp. 23749-23755.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 78

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) fur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst ct al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557). The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta-**glycosidases** from Sulfolobus solfataricus, Thermo-toga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at **pH** 7.4 and 105 degrees C. Although the enzyme had a half-life of greater than 60 h at 90 degrees C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at **100** degrees **C**. K-m and V-max values for the beta-mannosidase were determined to be 0.79 mM and 31.1 mu mol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the P.

furiosus beta-glucosidase (5.3 versus 4, 500 s(-1) mM(-1) with p-nitrophenyl-beta-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:95843 HCAPLUS
TITLE: Novel substrate specificity of a membrane-bound .beta.-**glycosidase** from the hyperthermophilic archaeon **Pyrococcus** horikoshii
AUTHOR(S): Matsui, I.; Sakai, Y.; Matsui, E.; Kikuchi, H.; Kawarabayasi, Y.; Honda, K.
CORPORATE SOURCE: National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki, Japan
SOURCE: FEBS Lett. (2000), 467(2,3), 195-200
CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A .beta.-**glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully expressed in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85.degree.C for 15 min. The optimum **pH** was 6.0 and the optimum temp. was over **100**.degree.**C**, resp. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90.degree.C (**pH** 6.0) was 15 h. BGPh has a novel substrate specificity with kcat/Km values high enough for hydrolysis of .beta.-D-Glcp derivs. with long alkyl chain at the reducing end and low enough for the hydrolysis of .beta.-linked glucose dimer more hydrophilic than aryl- or alkyl-.beta.-D-Glcp.

L20 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:590680 HCAPLUS
DOCUMENT NUMBER: 125:268847
TITLE: Comparison of a .beta.-glucosidase and a .beta.-mannosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus. Purification, characterization, gene cloning, and sequence analysis
AUTHOR(S): Bauer, Michael W.; Bylina, Edward J.; Swanson, Ronald V.; Kelly, Robert M.
CORPORATE SOURCE: Dep. Chem. Eng., North Carolina State Univ., Raleigh, NC, 27695-7905, USA
SOURCE: J. Biol. Chem. (1996), 271(39), 23749-23755
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two distinct exo-acting, .beta.-specific glycosyl hydrolases were purified to homogeneity from crude cell exts. of the hyperthermophilic archaeon **Pyrococcus** furiosus: a .beta.-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a .beta.-mannosidase. The .beta.-mannosidase and .beta.-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calcd. mol. masses of 59.0 and 54.6 kDa for the .beta.-mannosidase and the .beta.-glucosidase, resp. The .beta.-glucosidase gene was identical to that reported by Voorhorst et

al. (Voorhorst, W. [redacted] B., Eggen, R. I. L., Luesink, [redacted] J., and deVos, W. M. (1995) J. Bacte[redacted]l. 177, 7105-7111; GenBank acc[redacted]ion no. U37557). The deduced amino acid sequences showed homol. both with each other (46.5% identical) and with several other glycosyl hydrolases, including the .beta.-**glycosidases** from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the .beta.-mannosidase and the .beta.-glucosidase can both be classified as family 1 glycosyl hydrolases. In addn., the .beta.-mannosidase and .beta.-glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The .beta.-mannosidase showed optimal activity at **pH** 7.4 and 105.degree.C. Although the enzyme had a half-life of greater than 60 h at 90.degree.C, it is much less thermostable than the .beta.-glucosidase, which had a reported half-life of 85 h at **100**.degree.**C**. Km and Vmax values for the .beta.-mannosidase were detd. to be 0.79 mM and 31.1 .mu.mol para-nitrophenol released/min/mg with p-nitrophenyl-.beta.-D-mannopyranoside as substrate. The catalytic efficiency of the .beta.-mannosidase was significantly lower than that reported for the P. furiosus .beta.-glucosidase (5.3 vs. 4, 500 s-1 mM-1 with p-nitrophenyl-.beta.-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the .beta.-glucosidase, the primary role of the .beta.-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 15 OF 15 LIFESCI COPYRIGHT 2000 CSA
ACCESSION NUMBER: 97:345 LIFESCI
TITLE: Comparison of a beta -glucosidase and a beta -mannosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus: Purification, characterization, gene cloning, and sequence analysis
AUTHOR: Bauer, M.W.; Bylina, E.J.; Swanson, R.V.; Kelly, R.M.*
CORPORATE SOURCE: Dep. Chem. Eng., North Carolina State Univ., Raleigh, NC 27695-7905, USA
SOURCE: J. BIOL. CHEM., (1996) vol. 271, no. 39, pp. 23749-23755. ISSN: 0021-9258.
DOCUMENT TYPE: Journal
FILE SEGMENT: N; G; J
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Two distinct exo-acting, beta -specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a beta -glucosidase, corresponding to the one previously purified by Kengen et al., and a beta -mannosidase. The beta -mannosidase and beta -glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta -mannosidase and the beta -glucosidase, respectively. The beta -glucosidase gene was identical to that reported by Voorhorst et al. The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta -**glycosidases** from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the beta -mannosidase and the beta -glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta -mannosidase and beta -glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The beta -mannosidase showed

Journal code: EUH. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200006
ENTRY WEEK: 20000602

AB A beta-**glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully expressed in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85 degrees C for 15 min. The optimum **pH** was 6.0 and the optimum temperature was over **100** degrees **C**, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90 degrees C (**pH** 6.0) was 15 h. BGPh has a novel substrate specificity with k(cat)/K(m) values high enough for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of beta-linked glucose dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp.

L21 ANSWER 2 OF 6 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 1999327626 EMBASE
TITLE: Gene analysis and enzymatic properties of thermostable .beta.-**glycosidase** from **Pyrococcus** kodakaraensis KOD1.
AUTHOR: Ezaki S.; Miyaoku K.; Nishi K.-I.; Tanaka T.; Fujiwara S.; Takagi M.; Atomi H.; Imanaka T.
CORPORATE SOURCE: T. Imanaka, Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
SOURCE: Journal of Bioscience and Bioengineering, (1999) 88/2 (130-135).
Refs: 22
ISSN: 1389-1723 CODEN: JBBIF6
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A .beta.-**glycosidase** with broad substrate specificity was identified from a hyperthermophilic archaeon, **Pyrococcus** kodakaraensis KOD1. The gene encoding .beta.-**glycosidase** (Pk-gly) consists of 1449 nucleotides corresponding to a polypeptide of 483 amino acids. The protein showed similarity with other .beta.-**glycosidases** from family-1 glycosyl hydrolases, in particular, it showed high identity to .beta.-mannosidase from P. furiosus (55.7%), .beta.-**glycosidase** from Sulfolobus solfataricus (42.7%) and .beta.-glucosidase from P. furiosus (41.9%). The cloned gene was expressed in Escherichia coli and the recombinant protein was purified. The .beta.-**glycosidase** showed optimal activity at **pH** 6,5 and at an extremely high temperature of **100**.degree.**C**, and had a half-life of 18 h at 90.degree.C. The .beta.-**glycosidase** hydrolyzed various pNp-.beta.-glycopyranosides, with k(cat)/K(m) values in the order of pNp-.beta.-glucopyranoside dot left right equal to pNp-.beta.-mannopyranoside>pNp-.beta.-galactopyranoside>pNp-.beta.-xylopyranoside. pNp-.beta.-mannopyranoside was the substrate exhibiting the lowest K(m) value [0.254 mM] with a k(cat)/K(m) ratio comparable to that of pNp-.beta.-glucopyranoside. This substrate specificity was distinct from previously reported .beta.-**glycosidases**. We observed that the region in Pk-Gly corresponding to the fifth .alpha.-helix and .beta.-strand region of .beta.-**glycosidase**

from S. solfataricus which constitutes a large portion of the channel for substrate incorporation, displayed a chimeric structure, with the N-terminal region similar to .beta.-**glycosidases** and the C-terminal region similar to .beta.- mannosidases. An exo-type hydrolytic activity and transglycosylation activity were also observed towards cellooligomers.

L21 ANSWER 3 OF 6 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97196266 EMBASE
DOCUMENT NUMBER: 1997196266
TITLE: Cloning, sequencing, characterization, and expression of an extracellular .alpha.-amylase from the hyperthermophilic archaeon **Pyrococcus** furiosus in Escherichia coli and Bacillus subtilis.
AUTHOR: Jorgensen S.; Vorgias C.E.; Antranikian G.
CORPORATE SOURCE: G. Antranikian, Inst. of Biotechnology, Dept. of Technical Microbiology, Technical University Hamburg-Harhurg, Denickestr. 15, 21071 Hamburg, Germany
SOURCE: Journal of Biological Chemistry, (1997) 272/26 (16335-16342).
Refs: 27
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A gene encoding a highly thermostable extracellular .alpha.-amylase from the hyperthermophilic archaeon **Pyrococcus** furiosus was identified. The gene was cloned, sequenced, and expressed in Escherichia coli and Bacillus subtilis. The gene is 1383 base pairs long and encodes a protein of 461 amino acids. The open reading frame of the gene was verified by microsequencing of the recombinant purified enzyme. The deduced amino acid sequence is 25 amino acids longer at the N terminus than that determined by sequencing of the purified protein, suggesting that a leader sequence is removed during transport of the enzyme across the membrane. The recombinant .alpha.-amylase was biochemically characterized and shows an activity optimum at **pH** 4.5, whereas the optimun temperature for enzymatic activity is close to **100** .degree.**C**. .alpha.- Amylase shows sequence homology to the other known .alpha.-amylases and belongs to family 13 of glycosyl hydrolases. This extracellular .alpha.-amylase is not homologous to the subcellular .alpha.-amylase previously isolated from the same organism.

L21 ANSWER 4 OF 6 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 96394494 MEDLINE
DOCUMENT NUMBER: 96394494
TITLE: Comparison of a beta-glucosidase and a beta-mannosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus. Purification, characterization, gene cloning, and sequence analysis.
AUTHOR: Bauer M W; Bylina E J; Swanson R V; Kelly R M
CORPORATE SOURCE: Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Sep 27) 271 (39) 23749-55.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals

OTHER SOURCE: GENBANK-U60214
ENTRY MONTH: 199[redacted]

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557U37557). The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta-**glycosidases** from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at **pH** 7.4 and 105 degrees C. Although the enzyme had a half-life of greater than 60 h at 90 degrees C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at **100** degrees **C**. Km and Vmax values for the beta-mannosidase were determined to be 0.79 mM and

31.1 micromol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the P. furiosus beta-glucosidase (5.3 versus 4, 500 s-1 mM-1 with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L21 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1997:170983 BIOSIS
DOCUMENT NUMBER: PREV199799477586
TITLE: A new thermostable glucose-activated beta-glucosidase from the hyperthermophilic marine archaebacterium **Pyrococcus** abyssi: Purification and characterization.
AUTHOR(S): Ladrat, Christine (1); Alayse-Danet, Anne-Marie; Dietrich, Jacques; Barbier, George
CORPORATE SOURCE: (1) Dep. Environ. Profond, IFREMER, BP 70, 29280 Plouzane France
SOURCE: Journal of Marine Biotechnology, (1996) Vol. 4, No. 4, pp. 192-199.
ISSN: 0941-2905.
DOCUMENT TYPE: Article
LANGUAGE: English

AB A thermostable beta-glucosidase from the deep-sea hyperthermophilic archaebacterium **Pyrococcus** abyssi strain ST549 has been purified to apparent homogeneity and characterized. Production of beta-glucosidase by this strain was stimulated by the addition of cellobiose to the culture medium. This enzyme was homodimeric, each subunit having a molecular weight of 64 kDa. Its isoelectric point was 4.3. The enzyme showed maximal activity at **pH** 6 and **100** degree **C** and was highly thermostable. It exhibited different beta-**glycosidase**

activities and hydrolyzed short-chain oligosaccharides. Chemical modifications of amino acids indicated that no accessible sulfhydryl group was essential for activity and that histidine and tryptophan were probably involved in the maintenance of the integrity of the active site. In marked contrast to others presently known, the beta-glucosidase from the ST549 strain was activated by glucose and polyols. This property suggests specific interactions between the enzyme and polyhydric alcohols and the probable occurrence of a transglycosylation mechanism. This mechanism and potential applications are discussed.

L21 ANSWER 6 OF 6 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
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TITLE: Purification and characterization of an .alpha.-glucosidase from a hyperthermophilic archaebacterium, **Pyrococcus** furiosus, exhibiting a temperature optimum of 105 to 115.degree.C.
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SUMMARY LANGUAGE: English

AB **Pyrococcus** furiosus is a strictly anaerobic hyperthermophilic archaebacterium with an optimal growth temperature of about **100** .degree.**C**. When this organism was grown in the presence of certain complex carbohydrates, the production of several amylolytic enzymes was noted. These enzymes included an .alpha.-**glycosidase** that was located in the cell cytoplasm. This .alpha.-glucosidase has been purified 310-fold and corresponded to a protein band of 125 kilodaltons as resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The enzyme exhibited optimum activity at **pH** 5.0 to 6.0 and over a temperature range of 105 to 115.degree.C. Kinetic analysis conducted at 108.degree.C revealed hydrolysis of the substrate p-nitrophenyl-.alpha.-D-glucopyranoside (PNPG), methyl-.alpha.-D-glucopyranoside, maltose, and isomaltose. Trace activity was detected towards p-nitrophenyl-.beta.-D-glucopyranoside, and no activity could be detected towards starch or sucrose. Inhibition studies conducted at 108.degree.C with PNPG as the substrate and maltose as the inhibitor yielded a K(i) for maltose of 14.3 mM. Preincubation for 30 min at 98.degree.C in 100 mM dithiothreitol and 1.0 M urea had little effect on enzyme activity, whereas preincubation in 1.0% sodium dodecyl sulfate and 1.0 M guanidine hydrochloride resulted in significant loss of enzyme activity. Purified .alpha.-**glycosidase** from P. furiosus exhibited remarkable thermostability; incubation of the enzyme at 98.degree.C resulted in a half life of nearly 48 h.